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LINEAR SYNTHESIS OF THE METHYL GLYCOSIDES OF TETRA- AND PENTASACCHARIDE FRAGMENTS SPECIFIC FOR THE *Shigella flexneri* SEROTYPE 2a *O*-ANTIGEN[1]

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ABSTRACT

Starting from the known methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-α-L-rhamnopyranoside, the stepwise linear syntheses of methyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→ 3)-[α-D-glucopyranosyl-(1→ 4)]-α-L-rhamnopyranoside (**AB(E)C**, **4**), and methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→ 2)-α-L-rhamnopyranosyl-(1→ 3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (**DAB(E)C**, **5**) are described; these constitute the methyl glycosides of a branched tetra- and pentasaccharide fragments of the *O*-specific polysaccharide of *Shigella flexneri* serotype 2a, respectively. The chemoselective *O*-deacetylation at position 2_B and/or 2_A of key tri- and tetrasaccharide intermediates bearing a protecting group at position 2_C was a limiting factor. As such a step occurred once in the synthesis of **4** and twice in the synthesis of **5**, the regioselective introduction of residue **A** on a **B(E)C** diol precursor (**12**) and that of residue **D** on an **AB(E)C** diol precursor (**19**) was also attempted. In all cases, a trichloroacetimidate donor was involved. The latter pathway was found satisfactory for the construction of the target **4** using the appropriate tri-*O*-benzoyl rhamnosyl donor. However, attempted chain elongation of **12** using 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl trichloroacetimidate (**8**) resulted in an inseparable mixture which needed to be benzoylated to allow the isolation of the target tetrasaccharide. Besides, condensation of the corresponding tetrasaccharide acceptor and the *N*-acetylglucosaminyl donor was sluggish. As the target pentasaccharide was isolated in a poor yield, this route was abandoned.

INTRODUCTION

Shigella flexneri serotype 2a is a common infective agent in humans that is responsible for the endemic form of shigellosis, or bacillary dysentery. Field studies as well as studies on experimental models showed that protection against infection by this Gram negative bacterium is specific for the serotype of the strain.[2-4] The latter is defined by the structure of the *O*-specific polysaccharide (*O*-SP). It was suggested that immunization with protein conjugates of the *O*-SP of several enteropathogenic bacteria might offer protection against infection by the homologous strain.[5] Indeed, such an approach has been evaluated in the case of *Shigella flexneri* serotype 2a.[6,7] Nevertheless, optimal features for such conjugates are not well understood.

As part of a program aimed at the design of optimal vaccine conjugates based on the use of synthetic fragments of the *O*-SP, the study, at the molecular level, of the interaction between the bacterial antigen and homologous antibodies, was undertaken in our laboratory. For that reason, several oligosaccharides intended to elucidate the major conformational epitopes recognised by a panel of antibodies specific for this serotype were required in rather large quantities. Their synthesis is ongoing in the laboratory.

A **B** **C** **D**

2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→

(1↑4)

E α-D-Glc*p*

I

S. flexneri serotype 2a is defined by the branched pentasaccharide repeating unit[8,9] **I** of its *O*-SP, containing α-linked L-rhamnose and D-glucose together with β-linked *N*-acetyl-D-glucosamine as the monosaccharide constituents. As part of this project, we reported earlier[10] the synthesis of the methyl glycosides **1**, **2**, and **3** of the **EC**, **B(E)C** and **ECD** fragments, respectively. We describe herein the preparation of the tetra- (**AB(E)C**) and pentasaccharide (**DAB(E)C**) fragments. As before, they were synthesized as their methyl glycosides **4**, and **5**, to allow binding studies in solution. To our knowledge, compounds **4** and **5** are the first tetra- and pentasaccharides synthesized in this series.

α-D-Glc*p*-(1→4)-α-L-Rha*p*-OMe

1

α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-OMe

2

α-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-OMe

3

α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-OMe

4

β-D-GlcNAc*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-OMe

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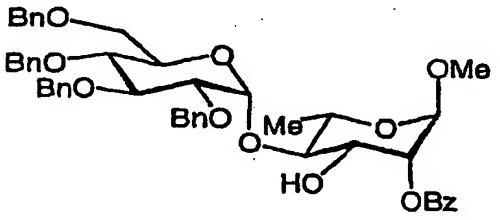
RESULTS AND DISCUSSION

As both the tetrasaccharide **4** and the pentasaccharide **5** were required, we reasoned that the most suitable approach was to combine heterofunctional monosaccharide intermediates in a stepwise manner. Besides, as the **EC** glycosidic bond corresponds to a 1,2-cis α-D-linkage, we anticipated that amongst all, it should be introduced first. Thus, the **EC** portion was constructed as described earlier[10] from the known 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl fluoride[11,12] and methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[13] to give, after appropriate transformation, the disaccharide acceptor **6** as the key precursor in the synthesis of the targets **4** and **5**.



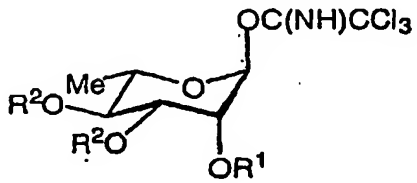
6

Synthesis of the tetrasaccharide 4.

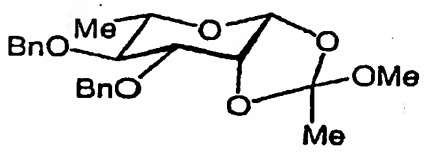
L-Rhamnose: A unit. Based on the experience gained in the *S. flexneri* serotype 5a series[14] as well as in the *S. flexneri* 2a series,[10] the tri-*O*-benzoylated trichloroacetimidate[14,15] **7** was used as a chain terminator precursor.

L-Rhamnose: B unit. Previous work had shown that trichloroacetimidates were suitable donors in the construction of the **BC** linkage. Based on this knowledge, the known 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl trichloroacetimidate[16,17] **(8)** was considered as a suitable precursor to residue **B**. Having permanent protecting groups at position 3 and 4 and bearing an acetyl group at position 2 which is selectively cleaved in the presence of both benzyl and benzoyl groups, compound **8** allows chain elongation

at position 2, as required. It was prepared in 7 steps from the commercially available L-rhamnose, using the orthoester intermediate **9** as described.[17]

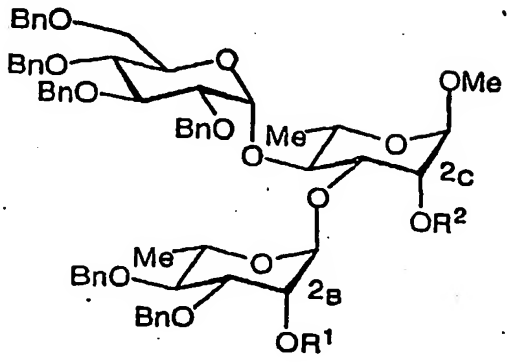


	R^1	R^2
7	Bz	Bz
8	Ac	Bn



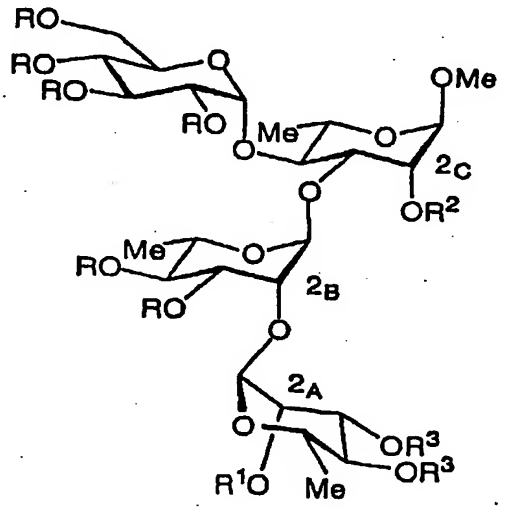
9

In analogy with previous work,[10] the monobenzoylated **EC** precursor **6** was condensed to the trichloroacetimidate **8** using diethyl ether as the solvent and a catalytic amount of trimethylsilyl triflate (TMSOTf) as the promoter. The glycosylation proceeded smoothly to afford the fully protected trisaccharide **10** in 97% yield, which was deacetylated at position 2_B. Although the chemoselective *O*-deacetylation of an *O*-benzoylated compound is a well-known process, this latter transformation was not straightforward and had to be optimised. Several procedures have been described in the literature to perform such a transformation. Conditions previously recommended include mildly basic medium using $Mg(OMe)_2$ in methanol,[18] DBU in methanol[19] or methanolic ammonia,[20] as well as acid catalysed methanolysis using methanolic hydrogen chloride in dichloromethane[21,22] or HBF_4 in diethyl ether/methanol.[23] As a general observation, acetolysis was preferred to transesterification or basic treatment. In our hands, the best results were obtained using the HBF_4 procedure which resulted in the alcohol **11** (65%) together with the starting **10** (13%), when performed under very mild conditions. The kinetics of the deprotection was totally in favour of the monohydroxylated product **11**. Although it was detected as a side-product in the reaction mixture, diol **12** was never present in amount large enough to be isolated. Anyhow, as the presence of multiple degradation products in the reaction mixture increased with time, the reaction had to be stopped before completion. Next, condensation of the **B(E)C** acceptor **11** with the trichloroacetimidate **7**, was performed under TMSOTf promotion as described above, to give the tetra-*O*-benzoylated tetrasaccharide **13** (95%). The all α-interglycosidic linkages in compound **13** was indicated by the $^1J_{C,H}$ heteronuclear coupling constants for residues **A**, **B**, **C** and **E**, which values were of 171, 171, 169 and 169 Hz, respectively. Conventional hydrogenolysis using palladium on charcoal gave the partially protected **14** (88%) and subsequent Zemplén debenzoylation gave the target tetrasaccharide **4** (90%).



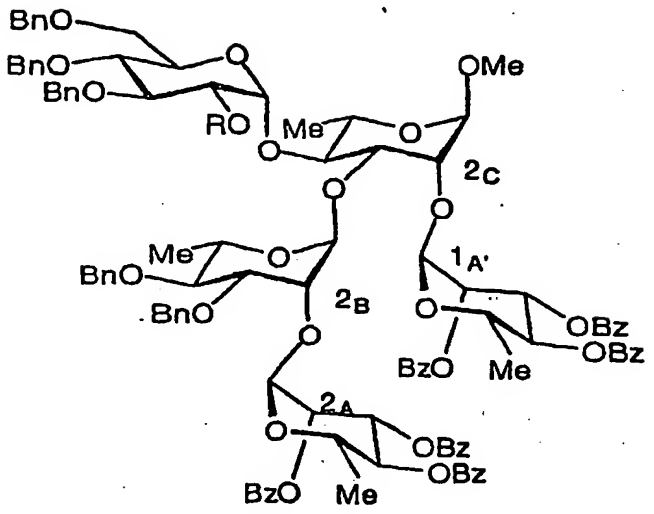
	R^1	R^2
10	Ac	Bz
11	H	Bz
12	H	H

In order to circumvent the chemoselective deacylation step, condensation of the chain terminator **7** and diol **12**, easily obtained upon Zemplén debenzoylation of **10** (95%), was attempted. Analogously to the conditions used with the benzoylated acceptor **11**, a rather large amount of TMSOTf catalyst (0.5 eq) was required in order for the



	R	R^1	R^2	R^3
13	Bn	Bz	Bz	Bz
14	H	Bz	Bz	Bz
15	Bn	Bz	H	Bz
16	Bn	Ac	Bz	Bn
17	Bn	H	Bz	Bn
18	Bn	Ac	H	Bn
19	Bn	H	H	Bn

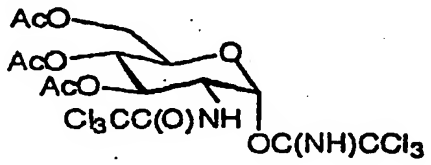
reaction to proceed smoothly. Unexpectedly, the kinetics of the condensation of **12** and **7** was slow compared to that of **11** and **7**, and degradation of **7** occurred first. The latter problem was overcome by portionwise addition of the donor, which resulted in the regioselective condensation product **15** in a satisfactory yield (69%). The 2_C-branched regioisomer was not isolated. However, the presence of a slight amount of the corresponding 2_B,2_C-di-branched pentasaccharide **20** was detected. The latter probably resulted from extensive glycosylation of the target **15**. Indeed, using a larger amount of donor and running the reaction at higher temperature (see experimental) resulted in the bis-condensation leading to **20** as the major product (78%, not optimized). The distortion of several signals in its ^{13}C NMR spectrum showed that compound **20** is sterically hindered. Basically, careful control of the portionwise addition of the donor **7** rendered this route to the target **15** satisfactory.



20

Synthesis of the pentasaccharide 5.

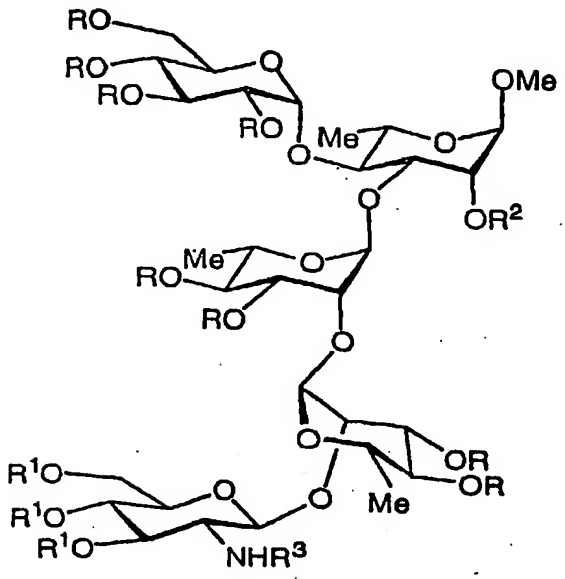
L-Rhamnose: A and B units. In the case of the construction of the target pentasaccharide **5**, residues **A** and **B** are involved in identical linkages, thus the trichloroacetimidate **7** was used as a precursor common to both residues.



21

***N*-Acetyl-D-glucosamine: D unit.** Our efforts to synthesize oligosaccharides representative of fragments of the *O*-antigen *of S. flexneri* serotype 5a have led us to test several D-glucosamine donors as potential precursors to residue **D**.[24] Variations concerned both the anomeric activating group and the *N*-protecting group. This study demonstrated that position 2_A was particularly poorly reactive, at least when involved in an analogous **DA** linkage. However, using the *N*-trichloroacetamide trichloroacetimidate donor[25] **21** in acetonitrile, the poor reactivity of the acceptor could be overcome. Thus, based on previous experience, **21** was used as a suitable precursor to residue **D** in the synthesis of **5**. It was prepared, as described, in 4 steps from D-glucosamine.[25]

The trisaccharide **11**, which was described previously in the synthesis of **4**, was also a crucial intermediate in the synthesis of the target **5**. When condensed in diethyl ether with the trichloroacetimidate donor **8** in the presence of a catalytic amount of TMSOTf as the promoter, **11** afforded the fully protected tetrasaccharide **16** (95%).



	R	R^1	R^2	R^3
22	Bn	Ac	Bz	$C(O)CCl_3$
23	Bn	H	H	H
24	Bn	H	H	Ac
25	Bn	Ac	H	$C(O)CCl_3$

Repeating the above described procedure, acetolysis of **16** was performed in the presence of HBF_4 to give monohydroxylated **17** (83%). The chemoselectivity of the deprotection step was higher at this stage than in the case of trisaccharide **10**. This was interpreted as position 2_A being more accessible than position 2_B when compared to their respective position 2_C. Next, condensation of **17** and the *N*-trichloroacetamide D-glucosaminyl donor **21** was performed in acetonitrile, using a catalytic amount of TMSOTf as the promoter. As expected, the reaction proceeded smoothly to give the fully protected pentasaccharide **22** in 84% yield. The $^1J_{C,H}$ coupling constant for residues **B** and **D** was not accessible at this stage, thus the β interglycosidic linkage for residue **D** was ascertained by the $^1J_{C,H}$ heteronuclear coupling constant for the glucosamine unit of the partially deprotected **24** (159 Hz). Values of 168-170 Hz were obtained for residues **A**, **B**, **C** and **E**, which confirmed their α-anomeric orientation. Deacylation of **22** under Zemplén conditions proved to be sluggish, resulting in the formation of an unknown side-product of mobility close to that of **24**. When deacylation of **22** was performed at rt in 1M methanolic sodium methoxide containing a slight amount of Et_3N, hardly any side-reaction occurred, and the intermediate **23** was isolated in a satisfactory yield (85%) probably following a two-step de-*N*-trichloroacetylation process, involving (i) intramolecular trans-acylation to HO-3 of residue **D**, and (ii) deacylation of the latter. Further *in situ* *N*-acetylation of **23** gave the target **24** (80%), which was hydrogenolyzed into the free pentasaccharide **5** (83%).

In an attempt to overcome the limitations caused by the chemoselectivity of the deacetylation steps, the regioselective condensation approach was attempted at two different stages. In analogy to the preparation of **15**, condensation of the trisaccharide diol **12** and the rhamnopyranosyl donor **8** was performed. The condensation product **18** could

not be isolated at this stage. It was isolated as the corresponding **16** (77%), obtained upon benzoylation of the contaminated **18**. Zemplén transesterification of the latter gave diol **19** (96%). Condensation of the glucosaminyl donor **21** and diol **19** was attempted using several reaction conditions (not described). Analogously to the condensation of the mono-*O*-benzoylated acceptor **17** and **21**, the use of TMSOTf as the promoter and CH_3CN as the solvent was the best combination. As observed above, the diol acceptor **19** was less reactive than the corresponding **18**. Even though TMSOTf (0.4 eq) was added portionwise, no real compromise between degradation and condensation of the starting materials was really reached. At best, the pentasaccharide **25** was extracted from the reaction mixture after benzoylation, which gave the fully protected **22**, in an estimated yield of 25%. Considering the poor yield of the condensation, this route was abandoned.

EXPERIMENTAL

General Methods. General experimental methods not referred to in this section were as described previously.[14] TLC on precoated slides of Silica Gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, dichloromethane-methanol; *B*, cyclohexane-ethyl acetate, *C*, cyclohexane-diethyl ether, *D*, water-acetonitrile, *E*, 2-propanol-ammonia-water, *F*, cyclohexane-diethyl ether-ethyl acetate. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in 4N aq H_2SO_4. In the NMR spectra, of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. Interchangeable assignments in the ^{13}C NMR spectra are marked with an asterisk in listing of signal assignments. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the *O*-SP and identified by a subscript in listing of signal assignments. Low-resolution mass spectra were obtained by either chemical ionisation (CIMS) using NH_3 as the ionising gas, by electrospray mass spectrometry (ESMS), or by fast atom bombardment mass spectrometry (FABMS).

Methyl (2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1 → 4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (10). A solution of the 2-*O*-benzoylated disaccharide acceptor[10] **6** (25.2 g, 31.4 mmol) and the trichloroacetimidate donor[17] **8** (24.9 g, 47.0 mmol) in anhydrous Et_2O (100 mL) was stirred at -50 °C for 20 min. TMSOTf (3.0 mL, 15.8 mmol) was added, and the mixture was stirred for 16 h while the cooling bath was slowly coming back to –5 °C. As no starting acceptor could be detected (solvent *B*, 7:3), the reaction mixture was neutralised with Et_3N, and concentrated to dryness. Column chromatography of the

crude material (solvent *B*, 9:1) gave the fully protected trisaccharide **10** (35.6 g, 97%) as a colourless foam, $[\alpha]_D$ +25° (*c* 1.0); ^{1}H NMR: δ 8.07-7.10 (m, 35H, Ph), 5.72 (dd, 1H, $J_{2,3}$ = 2.3 Hz, H-2_B), 5.40 (dd, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.06 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.03 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_B), 5.00-4.82 (m, 5H, OCH_2), 4.81 (bs, 1H, H-1_C), 4.67-4.36 (m, 7H, OCH_2), 4.18 (dd, 1H, $J_{3,4}$ = 8.2 Hz, H-3_C), 4.10 (m, 1H, H-5_E), 4.05 (dd, 1H, $J_{2,3}$ = 9.3, $J_{3,4}$ = 9.3 Hz, H-3_E), 3.92 (bd, 1H, $J_{6a,6b}$ = 9.1 Hz, H-$6a_E$), 3.81 (dd, 1H, $J_{5,6b}$ = 3.4 Hz, H-$6b_E$), 3.79-3.70 (m, 4H, H-4_E, 4_C, 5_C, 3_B), 3.66 (dq, 1H, $J_{4,5}$ = 9.5 Hz, H-5_B), 3.53 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2_E), 3.41 (s, 3H, OCH_3), 3.35 (dd, 1H, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4 Hz, H-4_B), 2.16 (s, 3H, C(=O)CH_3), 1.41 (d, 3H, $J_{5,6}$ = 5.6 Hz, H-6_C), and 1.01 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_B); ^{13}C NMR: δ170.0, 165.8 (C=O), 138.8-127.3 (Ph), 99.2 (bs, C-1_B, $J_{C,H}$ = 169 Hz), 98.2 (bs, C-1_E, $J_{C,H}$ = 170 Hz), 97.8 (C-1_C, $J_{C,H}$ = 170 Hz), 81.8 (C-3_E), 81.2 (C-2_E), 79.8 (C-4_B), 79.5 (C-3_C), 78.9 (C-4_C), 77.7 (C-4_E*), 77.3 (C-3_B*), 75.6, 75.1, 74.8, 73.9, 72.9 (5C, OCH_2), 71.9 (C-2_C), 71.6 (C-5_E), 70.5 (OCH_2), 68.6 (2C, C-2_B, 5_B), 68.5 (C-6_E), 67.1 (C-5_C), 55.1 (OCH_3), 21.2 (C(=O)CH_3), 18.7 (C-6_C), and 17.6 (C-6_B); CIMS for $C_{70}H_{76}O_{16}$ (*M*, 1172.51) *m/z* 1190.8 $[M+NH_4]^+$.

Anal. Calcd for $C_{70}H_{76}O_{16}$: C, 71.65; H, 6.53%. Found: C, 71.52; H, 6.56%.

Methyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (11). A 54% HBF_4 solution in Et_2O (1.5 mL, 20 mmol) was added at rt to a solution of the fully protected **10** (2.02 g, 1.72 mmol) in MeOH (35 mL). The reaction mixture was stirred at rt for 4 days. Although some starting material remained (solvent *B*, 7:3), in order to avoid side-reactions, the reaction was quenched at this stage upon addition of Et_3N. The mixture was concentrated, and the residue was taken up in CH_2Cl_2. The organic phase was washed with 5% aq $NaHCO_3$, water and satd aq NaCl, dried and concentrated. Chromatography of the residue (solvent *B*, 7:3) gave the starting material **10** (265 mg, 13%) as the first eluting product. Next the alcohol **11** was eluted (1.26 g, 65%) from the column as a colourless oil, $[\alpha]_D$ +21° (*c* 1.0); ^{1}H NMR: δ 8.08-6.90 (m, 35H, Ph), 5.32 (dd, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.1 Hz, H-2_C), 5.09 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 5.06 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1_B), 4.98 (d, 1H, J = 10.9 Hz, OCH_2), 4.77 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_C), 4.89-4.45 (m, 11H, OCH_2), 4.21 (bd, 1H, H-2_B), 4.14 (dd, 1H, $J_{3,4}$ = 8.7 Hz, H-3_C), 4.07 (m, 1H, H-5_E), 4.03 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3_E), 3.80 (m, 2H, H-5_C, 4_C), 3.69 (m, 3H, H-$6a_E$, $6b_E$, 5_B), 3.64 (dd, 1H, H-4_E), 3.60 (dd, 1H, H-3_B), 3.56 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2_E), 3.42 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-4_B), 3.40 (s, 3H, OCH_3), 2.81 (d, 1H, $J_{OH,2}$ = 2.9 Hz, OH-2_B), 1.40 (d, 3H, $J_{5,6}$ = 5.5 Hz, H-6_C), and 1.15 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B); ^{13}C NMR: δ 165.9 (C=O), 138.7-127.4 (Ph), 102.8 (C-1_B), 98.2 (C-1_E), 98.0 (C-1_C), 81.7 (C-3_E), 81.0 (C-2_E), 79.9 (C-4_B), 79.4 (C-4_C), 79.1 (C-3_B), 78.5 (C-3_C), 77.7 (C-4_E), 75.7,

75.1, 74.2, 73.9 (4C, OCH_2), 73.2 (2C, OCH_2, C-2_C), 71.8 (OCH_2), 71.2 (C-5_E), 68.5 (C-6_E), 68.4 (C-2_B), 68.3 (C-5_B), 67.4 (C-5_C), 55.0 (OCH_3), 18.5 (C-6_C), and 17.6 (C-6_B); CIMS for $C_{68}H_{74}O_{15}$ (M, 1330.5) *m/z* 1148.8 $[M+NH_4]^+$.

Anal. Calcd for $C_{68}H_{74}O_{15}$: C, 72.19; H, 6.59%. Found: C, 72.05; H, 6.65%.

Methyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (12). Methanolic 1M sodium methoxide was added dropwise, at rt, to a solution of the fully protected **10** (810 mg, 690 μmol) in a 9:1 MeOH:dichloromethane mixture (5.0 mL) until pH 10 was reached. The reaction mixture was stirred at rt for 48 h. After that time, only a single more polar product was present in the mixture (TLC, solvent *B*, 13:7). The mixture was neutralised with Amberlite IR-120 (H^+), filtered and concentrated. Chromatography of the residue (solvent *B*, 7:3) gave diol **12** (673 mg, 95%) as a colourless foam; $[\alpha]_D$ +7° (*c* 1.0), ^1H NMR: δ 7.36-7.10 (m, 30H, Ph), 5.06 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1_B), 5.02 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.97 (d, 1H, J = 10.9 Hz, OCH_2), 4.67 (bs, 1H, H-1_C), 4.87-4.57 (m, 9H, OCH_2), 4.47 (d, 2H, J = 12.2 Hz, OCH_2), 4.25 (dd, 1H, H-2_B), 4.03 (m, 1H, H-5_E), 3.99 (dd, partially overlapped, 1H, H-3_E), 3.96 (bs, 1H, H-2_C), 3.86 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 8.8 Hz, H-3_C), 3.84 (m, 1H, H-5_B), 3.77 (dd, 1H, $J_{2,3}$ = 3.6, $J_{3,4}$ = 8.2 Hz, H-3_B), 3.72 (m, 3H, H-5_C, $6a_E$, $6b_E$), 3.62 (dd, 1H, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.5 Hz, H-4_E), 3.55 (dd, 1H, H-2_E), 3.53 (dd, 1H, H-4_C), 3.51 (dd, 1H, H-4_B), 3.38 (s, 3H, OCH_3), 3.12 (d, 1H, $J_{OH,2}$ = 1.6 Hz, OH-2_B), 2.29 (bs, 1H, $J_{OH,2}$ = 2.8 Hz, OH-2_C), 1.49 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), and 1.34 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B); ^{13}C NMR: δ 165.9 (C=O), 138.7-127.7 (Ph), 102.1 (C-1_B), 100.0 (C-1_C), 98.3 (C-1_E), 81.5 (C-3_E), 81.2 (C-3_C), 81.0 (C-4_C*), 79.9 (C-4_B), 79.5 (C-2_E*), 79.1 (C-3_B), 77.6 (C-4_E), 75.6, 75.1, 74.8, 73.5, 73.1, 72.0 (6C, OCH_2), 71.2 (C-5_E), 70.7 (C-2_C), 69.0 (C-5_B), 68.4 (2C, C-2_B, 6_E), 66.8 (C-5_C), 54.1 (OCH_3), 18.5 (C-6_C), and 18.0 (C-6_B); CIMS for $C_{61}H_{70}O_{14}$ (M, 1026.5) *m/z* 1044.5 $[M+NH_4]^+$.

Anal. Calcd for $C_{61}H_{70}O_{14}$: C, 71.33; H, 6.87%. Found: C, 71.43; H, 7.00%.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (13). A solution of the trisaccharide acceptor **11** (2.15 g, 1.9 mmol) and the tri-*O*-benzoyl donor[14,15] **7** (1.48 g, 2.38 mmol) in anhydrous Et_2O (50 mL) was stirred at -50 °C for 15 min. TMSOTf (200 μL, 0.97 mmol) was added, and the mixture was stirred for 16 h while the cooling bath was slowly coming back to –5 °C. As no starting acceptor could be detected (solvent *B*, 7:3), the reaction mixture was neutralised with Et_3N, and concentrated under vacuum. Column chromatography of the crude material (solvent *C*, 3:1) gave the fully protected tetrasaccharide **13** (2.86 g, 95%) as a colourless foam; $[\alpha]_D$ +66° (*c* 1.0); ^1H NMR: δ 8.10-7.03 (m, 50H, Ph), 5.91 (dd, overlapped, 1H, H-2_A), 5.87 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-

3_A), 5.68 (d, 1H, $J_{3,4}$ = 9.7, $J_{4,5}$ = 9.7 Hz, H-4_A), 5.45 (dd, 1H, $J_{1,2}$ = 1.9, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.29 (bs, 1H, H-1_A), 5.10 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_B), 5.07 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.98-4.69 (m, 5H, OCH_2), 4.80 (bs, 1H, H-1_C), 4.63 (d, 2H, OCH_2), 4.55 (bs, 1H, H-2_B), 4.53-4.40 (m, 4H, OCH_2), 4.42 (m, overlapped, 1H, H-5_A), 4.32 (d, 1H, J = 12.0 Hz, OCH_2), 4.10 (m, 1H, H-3_C), 4.08 (m, 1H, H-5_E), 4.01 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3_E), 3.80 (m, 2H, H-5_C, 4_C), 3.74 (m, 2H, H-$6a_E$, $6b_E$), 3.70-3.55 (m, 4H, H-4_E, 3_B, 4_B, 5_B), 3.49 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2_E), 3.40 (s, 3H, OCH_3), 1.42 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.38 (d, 3H, $J_{5,6}$ = 5.4 Hz, H-6_C), and 1.11 (d, 3H, $J_{5,6}$ = 5.6 Hz, H-6_B); ^{13}C NMR: δ 165.9, 165.8, 165.4, 165.1 (4C, C=O), 138.5-127.1 (Ph), 101.2 (C-1_B, $J_{C,H}$ = 171 Hz), 99.5 (C-1_A, $J_{C,H}$ = 171 Hz), 97.8 (C-1_C, $J_{C,H}$ = 169 Hz), 97.7 (C-1_E, $J_{C,H}$ = 169 Hz), 81.8 (C-3_E), 80.9 (C-2_E), 79.8 (bs, C-3_C), 79.7 (C-4_B), 78.8 (C-3_B), 77.9 (bs, C-4_C), 77.5 (C-4_E), 75.9 (C-2_B), 75.6, 75.0, 74.9, 73.9, 73.0 (5C, OCH_2), 72.2 (C-2_C), 71.9 (C-4_A), 71.2 (C-5_E), 70.9 (OCH_2), 70.6 (C-2_A), 70.0 (C-3_A), 69.2 (C-5_B), 68.1 (C-6_E), 67.3 (C-5_C), 67.2 (C-5_A), 55.1 (OCH_3), 18.9 (C-6_C), 18.1 (C-6_A), and 17.7 (C-6_B); CIMS for $C_{95}H_{96}O_{22}$ (M, 1589.79) *m/z* 1607.9 $[M+NH_4]^+$.

Anal. Calcd for $C_{95}H_{96}O_{22}$: C, 71.77; H, 6.09%. Found: C, 71.68; H, 6.35%.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-α-L-rhamnopyranosyl-(1 → 3)-[α-D-glucopyranosyl)-(1 →4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (14). The fully protected tetrasaccharide **13** (1.07 g, 0.67 mmol) was dissolved in a 9:1 ethanol:acetic acid mixture (30 mL), treated with 10% Pd-C catalyst (1.13 g), and the mixture was stirred under a hydrogen atmosphere. After 24 h, TLC (solvent *A*, 19:1) showed the presence of one more polar major product. The suspension was filtered on a bed of Celite, and the filtrate was concentrated. To eliminate any residual trace of the catalyst, the residue was chromatographed on a short column of silica gel (solvent *A*, 97:3) to give **14** (621 mg, 88%) as a colourless foam, $[\alpha]_D$ +128° (*c* 1.0); ^{1}H NMR: δ 8.05-7.15 (m, 20H, Ph), 5.92 (bs, 1H, H-2_A), 5.79 (bd, 1H, H-3_A), 5.65 (dd, 1H, $J_{3,4}$ = 9.9, $J_{4,5}$ = 9.9 Hz, H-4_A), 5.43 (bs, 1H, H-2_C), 5.30 (bs, 1H, H-1_A), 5.15 (bs, 2H, H-1_E, 1_B), 4.79 (d, 1H, H-1_C), 4.46 (dq, 1H, $J_{4,5}$ = 8.9 Hz, H-5_A), 4.16 (bs, 1H, H-2_B), 4.10 (bd, 1H, $J_{3,4}$ = 9.9 Hz, H-3_C), 4.01 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4_C), 3.91 (m, 3H, H-$6a_E$, $6b_E$, 5_E), 3.80 (m, 3H, 3_B, 5_C, 3_E), 3.59 (m, 3H, 4_B, 4_E, 2_E), 3.50 (m, 1H, H-5_B), 3.29 (s, 3H, OCH_3), 2.69 (bs, 1H, OH), 1.35 (d, 3H, $J_{5,6}$ = 5.9 Hz, H-6_A), 1.29 (d, 3H, $J_{5,6}$ = 5.5 Hz, H-6_C), and 0.83 (d, 3H, $J_{5,6}$ = 5.1 Hz, H-6_B); ^{13}C NMR: δ166.1, 166.0, 165.9, 165.8 (C=O), 133.7-125.3 (Ph), 99.7 (C-1_B), 99.3 (C-1_A), 97.5 (2C, C-1_E, 1_C), 80.4 (C-2_B), 78.7 (C-3_C), 77.1 (C-3_E), 75.1 (bs, C-4_B*), 73.5 (C-2_E), 72.7 (C-4_C), 72.1 (C-2_C), 71.8 (C-4_A), 71.2 (C-3_A), 70.6 (2C, C-2_A, 5_E), 70.0 (C-4_E*), 69.6 (C-5_B), 67.5 (C-5_C), 67.1 (C-5_A), 61.4 (C-6_E), 55.5 (OCH_3), 18.8 (C-6_C), and 17.3 (2C, C-6_A, 6_B); CIMS for $C_{53}H_{60}O_{22}$ (M, 1049.04) *m/z* 1071.4 $[M+NH_4]^+$.

Anal. Calcd for $C_{53}H_{60}O_{22}+1H_2O$: C, 59.66; H, 5.86%. Found: C, 59.77; H, 5.93%. Compound **14** could not be obtained solvent free.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→ 4)]-α-L-rhamnopyranoside (15). A solution of the diol acceptor **12** (195 mg, 161 μmol) and the trichloroacetimidate donor[14,15] **7** (147 mg, 242 μmol) in anhydrous Et_2O (10 mL) was stirred at -50 °C for 20 min. TMSOTf (16 μL, 81 μmol) was added, and the mixture was stirred for 2 h while the cooling bath was slowly coming back to –30 °C. More donor **7** (100 mg, 164 μmol) was added, and the mixture was stirred for another 1 h while the cooling bath had reached 0 °C. Only very little **12** remained (solvent *F*, 10:7:3), and the reaction mixture was neutralised with Et_3N, then concentrated to dryness. Column chromatography of the crude material (solvent *F*, 83:12:5) gave the monohydroxylated tetrasaccharide **15** (187 g, 69%) as a colourless foam, together with a small amount of starting **12** (5 mg, 2.5%). Compound **15** had $[\alpha]_D$ +62° (*c* 1.0); ^{1}H NMR: δ 8.09-7.03 (m, 45H, Ph), 5.95 (dd, overlapped, 1H, H-2_A), 5.91 (dd, partially overlapped, 1H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.4 Hz, H-3_A), 5.70 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4_A), 5.34 (d, 1H, $J_{1,2}$ = 0.9 Hz, H-1_A), 5.09 (bs, 1H, H-1_B), 5.01 (d, 1H, J = 10.9 Hz, OCH_2), 4.95 (d, 1H, J = 10.9 Hz, OCH_2), 4.90 (d, 1H, H-1_E), 4.86-4.71 (m, 4H, OCH_2), 4.66 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1_C), 4.56 (bs, 1H, H-2_B), 4.58-4.33 (m, 6H; OCH_2), 4.38 (dq, overlapped, 1H, H-5_A), 4.05 (bs, 1H, H-2_C), 4.03 (bd, 1H, H-5_E), 3.96 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3_E), 3.92-3.83 (m, 2H, $J_{2,3}$ = 3.1 Hz, H-5_B, 3_C), 3.81-3.63 (m, 6H, H-3_B, 4_B, $6a_E$, $6b_E$, 5_C, 4_E), 3.50 (dd, 1H, J = 9.1 Hz, H-4_C), 3.45 (dd, 1H, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.8 Hz, H-2_E), 3.37 (s, 3H, OCH_3), 2.09 (d, 1H, $J_{3,4}$ = 3.9 Hz, OH-2_C), 1.43 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_B), 1.41 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A), and 1.35 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C); ^{13}C NMR: δ 165.9, 165.5, 165.1 (3C, C=O), 138.5-127.3 (Ph), 100.9 (C-1_B, $J_{C,H}$ = 167 Hz), 100.2 (C-1_C, $J_{C,H}$ = 170 Hz), 99.5 (C-1_A, $J_{C,H}$ = 171 Hz), 97.4 (C-1_E, $J_{C,H}$ = 168 Hz), 82.2 (C-3_C), 81.7 (C-3_E), 81.1 (C-2_E), 79.6 (C-4_B), 78.7 (C-3_B), 77.5 (C-4_E), 77.2 (C-4_C), 75.8 (C-2_B), 75.6, 75.0, 73.8, 73.0 (5C, OCH_2), 71.9 (C-4_A), 71.0 (C-5_E), 70.8 (OCH_2), 70.7 (C-2_A), 70.1 (C-2_C), 70.0 (C-3_A), 69.4 (C-5_B), 68.2 (C-6_E), 67.2 (C-5_A), 67.0 (C-5_C), 54.9 (OCH_3), 18.7 (C-6_C), 18.2 (C-6_A*), and 18.1 (C-6_B*); CIMS for $C_{88}H_{92}O_{21}$ (M, 1484.60) *m/z* 1502.7 $[M+NH_4]^+$.

Anal. Calcd for $C_{88}H_{92}O_{21}$: C, 71.14; H, 6.24%. Found: C, 71.12; H, 6.29%.

Methyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (4). A solution of the tetra-*O*-benzoyl tetrasaccharide **14** (510 mg, 0.49 mmol) in methanol (50 mL) was treated with 1M methanolic sodium methoxide until pH 10 was reached, and the solution was stirred at rt for 3 days. At this time only one product could be seen on TLC (solvent *E*, 7:1:2). After

neutralisation with Amberlite IR-120 (H^+), filtration, and evaporation of the solvent, purification of the crude product was achieved by reverse phase chromatography. The column was eluted with solvent *D* (gradient 100:0 → 97.5:2.5) to give, after lyophilization, the target tetrasaccharide **4** (276 mg, 90%), as a hygroscopic powder, $[\alpha]_D$ −13° (*c* 1.0, MeOH); ^{1}H NMR: δ 5.20 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1_E), 5.06 (bs, 1H, H-1_B), 4.96 (bs, 1H, H-1_A), 4.64 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1_C), 4.07 (m, 2H, H-2_A, 2_B), 4.03 (bs, 1H, H-2_C), 3.96-3.84 (m, 6H, H-5_E, 3_C, 5_B, 5_C, 3_A, $6a_E$), 3.82-3.71 (m, 5H, H-4_C, $6b_E$, 3_E, 3_B, 5_A), 3.54 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2_E), 3.48 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4_B), 3.45 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_A), 3.42 (m, 1H, H-4_E), 3.39 (s, 3H, OCH_3), 1.41 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_C), 1.29 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_A), and 1.28 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_B); ^{13}C NMR: δ 103.4 (C-1_A, $J_{C,H}$ = 170 Hz), 102.1 (bs, C-1_B, $J_{C,H}$ = 171 Hz), 101.3 (C-1_C, $J_{C,H}$ = 171 Hz), 98.6 (bs, C-1_E, $J_{C,H}$ = 172 Hz), 80.4 (C-2_B), 80.0 (bs, C-3_C), 76.0 (bs, C-4_C), 73.4 (C-3_E), 72.9 (C-4_B), 72.8 (C-4_A), 72.7 (C-5_E), 72.1 (C-2_E), 70.8 (3C, C-3_B, 2_C, 2_A), 70.5 (C-3_A), 70.4 (C-4_E), 70.2 (C-5_B), 70.0 (C-5_A), 69.4 (C-5_C), 61.4 (C-6_E), 55.8 (OCH_3), 18.8 (C-6_C), 17.6 (C-6_A*), 17.4 (C-6_B*); CIMS for $C_{25}H_{44}O_{18}$ (M, 632.25) *m/z* 650 $[M+NH_4]^+$.

Anal. Calcd for $C_{25}H_{44}O_{18}+3H_2O$: C, 43.73; H, 7.34%. Found: C, 43.56; H, 7.06%. Compound **4** could not be obtained solvent free.

Methyl (2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (16). (a) A solution of the 2-*O*-benzoyl trisaccharide acceptor[10] **11** (1.23 g, 1.09 mmol) and the trichloroacetimidate donor[17] **8** (900 mg, 1.7 mmol) in anhydrous Et_2O (50 mL) was stirred at -50 °C for 20 min. TMSOTf (115 μL, 0.55 mmol) was added, and the mixture was stirred for 16 h while the cooling bath was slowly coming back to −5 °C. As no starting acceptor could be detected (solvent *B*, 7:3), the reaction mixture was neutralised with Et_3N, then concentrated to dryness. Column chromatography of the crude material (solvent *B*, 9:1) gave the fully protected tetrasaccharide **16** (1.82 g, 95%) as a colourless foam.

(b) A solution of the diol acceptor **12** (208 mg, 172 μmol) and the trichloroacetimidate donor[17] **8** (137 mg, 259 μmol) in anhydrous Et_2O (15 mL) was stirred at -50 °C for 20 min. TMSOTf (17 μL, 86 μmol) was added, and the mixture was stirred for 2 h while the cooling bath was slowly coming back to −20 °C. More donor **8** (100 mg, 188 μmol) was added, and stirring went on for 1.5 h. As very little starting acceptor remained (solvent *F*, 10:7:3), the reaction mixture was neutralised with Et_3N, then concentrated to dryness. Column chromatography of the crude material (solvent *F*, 50:83:12) gave the contaminated monohydroxyl tetrasaccharide **18** (226 mg).

Benzoyl chloride (50 μL, 423 μmol) was added to a solution of the contaminated **18** (220 mg) in pyridine (2.0 mL), and the mixture was stirred at rt overnight. Methanol

was added, and after conventional treatment, the crude material was column chromatographed (solvent *B*, 9:1) to give the fully protected **16** (225 mg, 77% from **12**); $[\alpha]_D$ +31° (*c* 1.0); ^{1}H NMR: δ 8.07-7.10 (m, 45H, Ph), 5.55 (dd, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.0 Hz, H-2_A), 5.36 (dd, 1H, $J_{1,2}$ = 2.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.07 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 5.05 (bs, 1H, H-1_A), 4.99 (bs, 1H, H-1_B), 4.79 (bs, 1H, H-1_C), 4.98-4.45 (m, 14H, OCH_2), 4.40 (bs, 1H, H-2_B), 4.34 (d, 2H, OCH_2), 4.10-3.90 (m, 5H, H-3_C, 5_E, 3_E, 3_A, 5_A), 3.84-3.71 (m, 4H, H-4_C, 5_C, $6a_E$, $6b_E$), 3.68-3.55 (m, 3H, H-4_E, 3_B, 5_B), 3.49 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2_E), 3.44 (dd, 1H, $J_{3,4}$ = 9.7, $J_{4,5}$ = 9.7 Hz, H-4_A), 3.97 (s, 3H, OCH_3), 3.31 (dd, 1H, $J_{3,4}$ = 9.6, $J_{4,5}$ = 9.4 Hz, H-4_B), 2.00 (s, 3H, C(=O)CH_3), 1.37 (d, 6H, $J_{5,6}$ = 6.1 Hz, H-6_A, 6_C), and 0.99 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B); ^{13}C NMR: δ 169.9, 165.8 (C=O), 138.8-127.2 (Ph), 104.7 (C-1_E, $J_{C,H}$ = 168 Hz), 101.0 (C-1_A, $J_{C,H}$ = 171 Hz), 99.4 (C-1_B, $J_{C,H}$ = 169 Hz), 97.8 (C-1_C, $J_{C,H}$ = 169 Hz), 82.3 (C-3_E), 81.8 (C-2_E), 81.0 (C-4_A), 80.1 (C-4_B), 79.6 (bs, C-3_C), 78.9 (C-3_B), 77.9 (bs, C-4_C), 77.7 (C-3_A), 77.6 (C-4_E), 75.6, 75.3, 75.0 (3C, OCH_2), 74.8 (C-2_B), 74.5, 73.9, 72.9 (3C, OCH_2), 72.4 (C-2_C), 71.7 (OCH_2), 71.3 (C-5_E), 70.7 (OCH_2), 69.1 (C-2_A), 68.8 (C-5_B), 68.2 (2C, C-5_A, 6_E), 67.3 (C-5_C), 55.1 (OCH_3), 21.0 (C(=O)CH_3), 18.8 (C-6_C*), 18.4 (C-6_A*), and 17.7 (C-6_B); CIMS for $C_{91}H_{102}O_{19}$ (M, 1498.70) *m/z* 1517.0 $[M+NH_4]^+$.

Anal. Calcd for $C_{91}H_{102}O_{19}$: C, 72.88; H, 6.85%. Found: C, 72.89; H, 6.73%.

Methyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→ 4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (17). A 54% HBF_4 solution in Et_2O (1.7 mL) was added dropwise, at rt, to a solution of the fully protected **16** (890 mg, 0.59 mmol) in MeOH (40 mL). The reaction mixture was stirred at rt for 5 days. Although some starting material remained (solvent *B*, 7:3), the reaction was quenched at this stage upon addition of Et_3N. The mixture was concentrated, and the residue was taken up in CH_2Cl_2. The organic phase was washed with 5% aq $NaHCO_3$, water and satd aq NaCl, dried and concentrated. Column chromatography of the residue (solvent *B*, 9:1) gave the alcohol **17** (720 mg, 83%) as a colourless foam; $[\alpha]_D$ +23° (*c* 1.0); ^{1}H NMR: δ 8.10-6.88 (m, 45H, Ph), 5.39 (dd, 1H, $J_{1,2}$ = 2.1, $J_{2,3}$ = 2.8 Hz, H-2_C), 5.12 (bs, 1H, H-1_A), 5.10 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1_E), 5.08 (bs, 1H, H-1_B), 4.82 (bs, 1H, H-1_C), 5.02-4.64 (m, 11H, OCH_2), 4.54-4.32 (m, 6H, 5 OCH_2, H-2_B), 4.13-4.09 (m, 3H, H-3_C, 5_C, 5_E), 4.05 (dd, 1H, $J_{2,3}$ = 9.4, $J_{3,4}$ = 9.4 Hz, H-3_E), 3.94 (dq, 1H, $J_{4,5}$ = 9.4, $J_{5,6}$ = 6.3 Hz, H-5_A), 3.87 (dd, 1H, $J_{2,3}$ = 3.1, $J_{3,4}$ = 9.2 Hz, H-3_A), 3.85-3.73 (m, 5H, H-4_C, $6a_E$, $6b_E$, 4_E, 2_A), 3.62 (dd, 1H, H-3_B), 3.58 (dq, 1H, H-5_B), 3.51 (dd, 1H, H-4_A), 3.50 (dd, 1H, H-2_E), 3.40 (s, 3H, OCH_3), 3.28 (dd, 1H, $J_{3,4}$ = 9.3, $J_{4,5}$ = 9.3 Hz, H-4_B), 1.40 (d, 6H, H-6_A, 6_C), and 1.05 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_B); ^{13}C NMR: δ 165.8 (C=O), 138.8-127.3 (Ph), 101.1 (bs, C-1_B), 101.0 (C-1_A), 97.8 (C-1_C), 97.7 (C-1_E), 81.8 (C-3_E), 80.9 (C-2_E), 80.2 (C-4_A), 79.9 (C-4_B),

79.5 (2C, C-3_A, 3_C), 78.8 (C-3_B), 77.8 (bs, C-4_C), 77.6 (C-4_E), 75.7, 75.4, 75.2 (3C, OCH_2), 74.9 (C-2_B), 74.4, 73.9, 73.1 (3C, OCH_2), 72.5 (C-2_C), 72.1 (OCH_2), 71.2 (C-5_E), 70.7 (OCH_2), 68.8 (C-5_C), 68.7 (C-5_B), 68.2 (C-6_E), 68.0 (C-5_A), 67.4 (C-2_A), 55.1 (OCH_3), 18.9 (C-6_C*), 18.5 (C-6_A*), and 17.8 (C-6_B); CIMS for $C_{88}H_{96}O_{19}$ (M, 1456.65) *m/z* 1475.0 $[M+NH_4]^+$.

Anal. Calcd for $C_{88}H_{96}O_{19}$: C, 72.51; H, 6.64%. Found: C, 72.57; H, 6.53%.

Methyl (3,4-Di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→ 4)]-α-L-rhamnopyranoside (19). Methanolic 1M sodium methoxide was added dropwise, at rt, to a solution of the fully protected **16** (1.41 g, 941 μmol) in a 17:3 MeOH:dichloromethane mixture (6.0 mL) until pH 10 was reached. The reaction mixture was stirred at rt for 48 h. After that time, only one single more polar product was present in the mixture (TLC, solvent *B*, 7:3). The mixture was neutralised with Amberlite IR-120 (H^+), filtered and concentrated. Chromatography of the residue (solvent *B*, 17:3) gave diol **19** (1.2 g, 96%) as a colourless foam; $[\alpha]_D$ +3° (*c* 1.0), ^1H NMR: δ 7.40-7.13 (m, 40H, Ph), 5.11 (bs, 1H, H-1_A), 5.02 (bs, 1H, H-1_B), 4.96 (d, 1H, J = 10.9 Hz, OCH_2), 4.93-4.84 (m, 5H, 4 OCH_2, H-1_E), 4.64 (bs, 1H, H-1_C) 4.75-4.10 (m, 11H, OCH_2), 4.39 (bd, partially overlapped, 1H, H-2_B), 4.09 (bd, 1H, H-2_A), 4.03 (m, 1H, H-5_E), 4.01-3.86 (m, 4H, H-2_C, 3_E, 5_A, 3_A), 3.81-3.67 (m, 7H, H-3_C, $6a_E$, $6b_E$, 5_B, 4_E, 3_B, 5_C), 3.51 (dd, 1H, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4 Hz, H-4_A), 3.47 (dd, 1H, $J_{3,4}$ = 9.3, $J_{4,5}$ = 9.7 Hz, H-4_C), 3.45 (dd, 1H, $J_{1,2}$ = 3.9, $J_{2,3}$ = 10.1 Hz, H-2_E), 3.39 (dd, 1H, H-4_B), 3.36 (s, 3H, OCH_3), 1.35 (d, 6H, $J_{5,6}$ = 6.2 Hz, H-6_A, 6_C), and 1.29 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B); ^{13}C NMR: δ 138.8-127.3 (Ph), 100.9 (C-1_A), 100.6 (C-1_B), 100.1 (C-1_C), 97.4 (C-1_E), 81.7 (2C, C-3_C, 3_E), 81.1 (C-2_E), 80.2 (C-4_A), 79.8 (C-4_B), 79.5 (C-3_A), 78.7 (C-4_E), 77.6 (C-3_B), 77.3 (bs, C-4_C), 75.6, 75.5, 75.4, 75.2 (4C, OCH_2), 74.7 (C-2_B), 73.8, 73.1, 72.0 (3C, OCH_2), 71.0 (C-5_E), 70.4 (OCH_2), 69.9 (C-2_C), 69.1 (C-5_B), 68.8 (C-2_A), 68.2 (C-6_E), 68.0 (C-5_A), 66.9 (C-5_C), 54.9 (OCH_3), 18.8 (C-6_C*), and 18.3 (2C, C-6_A*, C-6_B); CIMS for $C_{82}H_{96}O_{17}$ (M, 1352.65) *m/z* 1370.8 $[M+NH_4]^+$.

Anal. Calcd for $C_{82}H_{96}O_{17}$: C, 72.76; H, 7.15%. Found: C, 72.73; H, 7.13%.

Methyl (2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→ 4)]-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→ 2)]-α-L-rhamnopyranoside (20). Compounds **12** (428 mg, 355 μmol) and **7** (323 mg, 532 μmol) in anhydrous Et_2O (20 mL) were treated with TMSOTf (35 μL, 177 μmol) as described for the preparation of **15**. After 2 h, when the bath temperature had reached –25 °C, more donor was added (345 mg, 567 μmol) and the mixture was stirred for another 1.5 h, to reach 15 °C. At this stage, TLC (solvent *F*, 10:7:3) showed that most of the intermediate **15** had turned into a

less polar product. The mixture was processed as described above to give, after repeated column chromatography, (solvent *F*, 83:12:15) the pentasaccharide **20** as a colourless foam (548 mg, 78%); $[\alpha]_D$ +72° (*c* 1.0); relevant ^{1}H NMR data: δ 8.15-6.96 (m, 60H, Ph), 5.95 (dd, 1H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, H-$3_{A'}$), 5.91 (bs, partially overlapped, 1H, H-2_A), 5.89 (dd, partially overlapped, 1H, $J_{2,3}$ = 3.2 Hz, H-3_A), 5.83 (bs, 1H, H-$2_{A'}$), 5.76 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-$4_{A'}$), 5.66 (dd, 1H, $J_{3,4}$ = 9.6, $J_{4,5}$ = 9.6 Hz, H-4_A), 5.38 (bs, 1H, H-$1_{A'}$), 5.34 (bs, 1H, H-1_A), 5.20 (bd, 2H, H-1_E, 1_B), 4.90 (d, 1H, H-1_E), 4.85 (bs, partially overlapped, 1H, H-1_C), 3.60 (dd, 1H, $J_{1,2}$ = 3.2, $J_{2,3}$ = 9.6 Hz, H-2_E), 3.42 (s, 3H, OCH_3), and 1.48-1.37 (m, 12H, H-6_A, 6_B, 6_C, $6_{A'}$); relevant ^{13}C NMR data: δ 165.9-165.0 (6C, C=O), 138.6-127.4 (Ph), 102.0 (bs, C-1_B), 99.5 (C-1_A), 99.3 (C-1_C), 99.0 (bs, C-$1_{A'}$), 96.7 (bs, C-1_E), 67.9 (C-6_E), 55.0 (OCH_3), and 19.2, 18.5, 18.2, 17.9 (4C, C-6_A, $6_{A'}$, 6_B, 6_C); FABMS for $C_{115}H_{114}O_{28}$ (M, 1943.7) *m/z* 1966.7 $[M+Na]^+$.

Anal. Calcd for $C_{115}H_{114}O_{28}$: C, 71.05; H, 5.91%. Found: C, 71.11; H, 5.99%.

Methyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 →2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1 → 2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (22). TMSOTf (17 μL, 86 μmol) was added, at 0 °C, to a solution of trichloroacetimidate donor[25] **21** (386 mg, 0.65 mmol) and tetrasaccharide acceptor **17** (631 mg, 0.43 mmol) in anhydrous acetonitrile (5 mL). The mixture was stirred for 2 h at this temperature. At this time, TLC (solvent *B*, 7:3) showed that no starting acceptor remained. Et_3N was added, and the reaction mixture was concentrated. Chromatography (solvent *B*, 4:1) of the residue gave the fully protected pentasaccharide **22** (685 mg, 84%) as a colourless foam; $[\alpha]_D$ +13° (*c* 1.0); ^{1}H NMR: δ 8.06-7.11 (m, 45H, Ph), 6.83 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH), 5.36 (dd, 1H, $J_{1,2}$ = 2.3, $J_{2,3}$ = 2.8 Hz, H-2_C), 5.12 (bs, 1H, H-1_A), 5.07 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 9.7 Hz, H-3_D), 4.98 (d, partially overlapped, 1H, H-1_E), 4.93 (m, 2H, H-4_D, 1_B), 4.89 (d, partially overlapped, 1H, H-1_D), 4.83 (bs, 1H, H-1_C), 4.98-4.42 (m, 16H, OCH_2), 4.46 (dd, partially overlapped, 1H, H-2_B), 4.16 (bs, 1H, H-2_A), 4.05-3.91 (m, 8H, H-3_C, 3_E, $6a_D$, 5_D, 2_D, 3_A, $6a_E$, 5_A), 3.82-3.72 (m, 4H, H-4_E, $6b_D$, 5_C, $6b_E$), 3.69 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-4_C), 3.65 (dd, 1H, H-3_B), 3.57 (dq, 1H, $J_{4,5}$ = 9.4 Hz, H-5_B), 3.49-3.40 (m, 3H, H-2_E, 4_A, 5_E), 3.39 (s, 3H, OCH_3), 3.28 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-4_B), 2.03 (s, 6H, 2 C(=O)CH_3), 1.75 (s, 3H, C(=O)CH_3), 1.37 (d, 6H, H-6_A, 6_C), and 1.02 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_B); ^{13}C NMR: δ 170.6, 170.4, 169.3, 165.8(OC(=O)), 161.8 (NC(=O)), 138.8-127.0 (Ph), 101.1 (C-1_D, $J_{C,H}$ not determined), 101.0 (bs, C-1_B, $J_{C,H}$ not determined), 100.7 (C-1_A, $J_{C,H}$ = 171 Hz), 98.1 (C-1_E, $J_{C,H}$ = 169 Hz), 97.8 (C-1_C, $J_{C,H}$ = 170 Hz), 92.2 (CCl_3), 81.8 (C-3_E), 81.3 (2C, C-2_E, 4_A), 80.1 (C-4_B), 80.0 (bs, C-3_C), 79.7 (C-3_A), 78.7 (C-4_C), 78.1 (C-3_B), 77.4 (C-4_E), 76.1 (C-2_A), 75.6, 75.5, 75.0 (3C, OCH_2), 74.5 (C-2_B), 74.3, 73.9, 73.8, 72.8

(4C, OCH_2), 72.6 (C-3_D), 72.2 (C-2_C), 72.1 (C-5_E), 71.5 (C-5_D), 70.0 (OCH_2), 68.6 (C-5_B), 68.4 (C-5_A), 68.1 (C-4_D), 68.0 (C-6_E), 67.1 (C-5_C), 61.7 (C-6_D), 55.8 (C-2_D), 55.1 (OCH_3), 20.7, 20.5 (3C, C(=O)CH_3), 18.7 (C-6_A*), 18.3 (C-6_C*), and 17.8 (C-6_B); FABMS for $C_{102}H_{112}Cl_3NO_{27}$ (M, 1887.65) *m/z* 1911.65 $[M+Na]^+$.

Anal. Calcd for $C_{102}H_{112}Cl_3NO_{27}$: C, 64.81; H, 5.97; N, 0.74%. Found: C, 64.81; H, 6.12; N, 0.71%.

Methyl (2-Amino-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→ 3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-α-L-rhamnopyranoside (23). Triethylamine (1.2 mL, 8.7 mmol) was added to a solution of the fully protected pentasaccharide 22 (309 mg, 164 μmol) in methanolic 1M sodium methoxide (10 mL), and the mixture was stirred at rt overnight. At this time TLC (solvent *A*, 19:1) showed that the starting material had been transformed into a more polar product, which reacted with ninhydrin. The mixture was cooled to 0 °C, and 5% aq HCl was added dropwise until the solution reached neutral pH. The resulting solution was concentrated, and coevaporated repeatedly with toluene and cyclohexane. The residue was column chromatographed (solvent *A*; 49:1) to give the amino derivative 23 (211 mg, 85%) as a colourless foam; $[\alpha]_D$ +5° (*c* 1.0); ^1H NMR: δ 7.36-7.00 (m, 40H, Ph), 5.28 (bs, 1H, H-1_A), 4.91 (bs, 1H, H-1_B), 4.75 (d, overlapped, 1H, H-1_E), 4.63 (bs, 1H, H-1_C), 4.95-4.41 (m, 15H, OCH_2), 4.26 (d, 2H, OCH_2, H-2_B), 4.10 (m, 2H, H-2_A, 1_D), 3.97-3.58 (m, 13H, H-3_A, 3_E, 2_C, 5_E, 5_A, $6a_E$, 5_B, 3_B, $6b_E$, 3_C, 4_E, $6a_D$, 5_C), 3.52-3.31 (m, 9H, H-2_E, 4_A, $6b_D$, 4_B, 4_D, 4_C, OCH_3), 3.19 (dd, partially overlapped, 1H, H-3_D), 3.11 (m, 1H, H-5_D), 2.53 (bdd, 1H, $J_{1,2} = J_{2,3} = 8.3$ Hz, H-2_D), 1.39 (d, 3H, $J_{5,6} = 6.1$ Hz, H-6_A), 1.35 (d, 3H, $J_{5,6} = 6.4$ Hz, H-6_C), and 1.32 (d, 3H, H-6_B); ^{13}C NMR: δ 138.7-127.3 (Ph), 106.1 (C-1_D), 101.8 (C-1_A), 100.1 (C-1_B), 100.1 (C-1_C), 98.4 (C-1_E), 82.2 (C-4_E), 81.6 (C-3_E), 81.3 (C-2_E), 80.3 (C-4_A), 80.1 (C-4_B), 78.8 (3C, C-4_C, 3_C, 3_A), 77.6 (C-2_A), 77.1 (C-3_B), 76.7 (C-3_D), 76.0 (C-5_D), 75.6, 75.5, 75.4 (3C, OCH_2), 75.0 (C-2_B), 74.9 (OCH_2), 73.8, 72.7, 72.1 (3C, OCH_2), 71.4 (C-5_E), 70.7 (OCH_2), 70.6 (C-4_D), 70.0 (C-2_C), 69.2 (C-5_B), 68.5 (C-5_A), 67.4 (C-6_E), 66.7 (C-5_C), 62.0 (C-6_D), 57.4 (C-2_D), 54.9 (OCH_3), 18.3 (2C, C-6_C, 6_A), and 17.8 (C-6_B); FABMS for $C_{87}H_{103}NO_{22}$ (M, 1513.77) *m/z* 1536.9 $[M+Na]^+$.

Anal. Calcd for $C_{87}H_{103}NO_{22}$: C, 68.98; H, 6.85; N, 0.92%. Found: C, 68.92; H, 6.93; N, 0.90%.

Methyl (2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→ 2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (24). (a) 1M methanolic sodium methoxide (3 mL) was added dropwise to a solution of the fully protected pentasaccharide 22 (201 mg, 106 μmol) in methanol (5 mL). Triethylamine

(230 μL, 1.67 mmol) was added to the mixture, which was stirred at rt for 4 days. At this time TLC (solvent *A*, 19:1) showed that the starting material had been transformed into a major more polar product which reacted with ninhydrin, namely the amino derivative **23**. The reaction mixture was cooled to 0 °C and acetic anhydride was added dropwise until neutral pH was reached. At this stage, more acetic anhydride (250 μL) was added, and the reaction was stirred at rt for 1 h. The mixture was concentrated under vacuum, coevaporated repeatedly with toluene and cyclohexane, and the residue was taken up in CH_2Cl_2. After conventional treatment, column chromatography (solvent *A*, 97:3) of the residue gave the tetraol **24** (132 mg, 80%) as a colourless foam.

(b) Acetic anhydride (500 μL, 5.3 mmol) was added dropwise, at 0 °C, to a solution of the amino derivative **23** (302 mg, 0.2 mmol). TLC (solvent *A*, 19:1) showed the total conversion of **23** into one less polar product. Volatiles were evaporated and coevaporated repeatedly with toluene and cyclohexane. Column chromatography of the residue gave the acetamido derivative **24** (284 mg, 92%); $[\alpha]_D$ +6° (*c* 1.0); ^{1}H NMR: δ 7.44-7.07 (m, 46H, Ph, NH), 6.74 (bs, 1H, OH), 6.52 (bs, 1H, OH), 5.25 (bs, 1H, H-1_A), 4.91 (bs, 1H, H-1_B), 4.72 (d, partially overlapped, 1H, H-1_E), 4.64 (bs, 1H, H-1_C), 4.96-4.59 (m, 12H, OCH_2), 4.49 (d, 2H, OCH_2), 4.39 (m, 3H, H-2_B, 2 OCH_2), 4.31 (d, partially overlapped, 1H, H-1_D), 4.11 (dd, 1H, H-2_A), 4.00 (dd, 1H, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.6 Hz, H-3_A), 3.99 (dd, overlapped, 1H, H-2_C), 3.98-3.86 (m, 3H, H-3_E, 5_E, 5_A), 3.83 (dq, 1H, $J_{4,5}$ = 9.2, $J_{5,6}$ = 6.0 Hz, H-5_B), 3.78-3.66 (m, 6H, H-$6a_E$, $6b_E$, 3_B, 3_C, 4_E, $6a_D$), 3.62 (dq, 1H, $J_{4,5}$ = 9.2, $J_{5,6}$ = 6.2 Hz, H-5_C), 3.48-3.41 (m, 4H, H-$6b_D$, 4_B, 4_A, 2_E), 3.39-3.30 (m, 8H, H-3_D, 2_D, 5_D, 4_C, 4_D, OCH_3), 2.03 (d, 1H, J = 2.9 Hz, OH), 1.74 (s, 1H, OH), 1.53 (s, 3H, C(=O)CH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.35 (d, 3H, H-6_B), and 1.34 (d, 3H, H-6_C); ^{13}C NMR: δ 173.7 (NC(=O)), 138.8-127.5 (Ph), 103.3 (C-1_D, $J_{C,H}$ = 159 Hz), 101.0 (2C, C-1_B, 1_A, $J_{C,H}$ = 169 Hz), 100.1 (C-1_C, $J_{C,H}$ = 170 Hz), 98.2 (C-1_E, $J_{C,H}$ = 168 Hz), 82.3 (C-3_C), 81.6 (C-3_E), 81.4 (C-4_B*), 81.2 (C-4_A*), 80.0 (C-2_E*), 79.9 (C-3_A), 79.0 (C-2_A), 78.8 (C-3_B), 78.5 (C-4_C), 77.2 (C-4_E), 77.1 (C-3_D), 75.9 (C-4_D), 75.8, 75.6, 75.5, 75.0, 74.3 (5C, OCH_2), 74.1 (C-2_B), 73.8, 72.8 (2C, OCH_2), 71.9 (C-5_D), 71.3 (C-5_E), 70.7 (OCH_2), 69.9 (C-2_C), 69.3 (C-5_B), 68.4 (C-5_A), 67.8 (C-6_E), 66.7 (C-5_C), 62.4 (C-6_D), 59.0 (C-2_D), 54.9 (OCH_3), 22.3 (C(=O)CH_3), 18.5 (C-6_C*), 18.3 (C-6_B*), and 17.8 (C-6_A); FABMS for $C_{89}H_{105}NO_{23}$ (M, 1555.71) *m/z* 1556.8 $[M+H]^+$.

Anal. Calcd for $C_{89}H_{105}NO_{23}$: C, 68.67; H, 6.80; N, 0.90%. Found: C, 68.56; H, 6.86; N, 0.91%.

Methyl 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranoside (5). The benzylated pentasaccharide **24** (205 mg, 132 μmol) was dissolved in a 9:1 ethanol:acetic acid mixture (10 mL), treated with 10% Pd-C

catalyst (500 mg), and the suspension was stirred at rt for 4 days, under a hydrogen atmosphere. At this time TLC (solvent *H*, 7:1:2) showed that the starting material had been transformed into a major more polar product. The suspension was filtered on a bed of Celite, and the filtrate was concentrated. Reverse phase chromatography (solvent *G*, gradient) of the residue, followed by lyophilization, gave the target pentasaccharide **5** (91 mg, 83%) as a colourless foam; $[\alpha]_D$ –6° (*c* 1.0); ^{1}H NMR: δ 5.19 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1_E), 5.13 (bs, 1H, H-1_A), 5.02 (bs, 1H, H-1_B), 4.67 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 4.63 (bs, 1H, $J_{1,2}$ = 2.1 Hz, H-1_C), 4.13 (bs, 1H, H-2_A), 4.08 (bs, 1H, H-2_B), 4.02 (bs, 1H, H-2_C), 3.98-3.67 (m, 14H, H-5_E, 3_C, 3_B, 5_C, $6a_D$, $6b_D$, $6a_E$, 5_B, 3_A, $6b_E$, 4_C, 5_A, 3_E, 2_D), 3.57-3.41 (m, 6H, H-3_D, 2_E, 4_B, 4_D, 5_D, 4_E), 3.39 (s, 3H, OCH_3), 3.33 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4_A), 2.04 (s, 3H, C(=O)CH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6_C), and 1.27 (d, 6H, $J_{5,6}$ = 6.2 Hz, H-6_A, 6_B); ^{13}C NMR: δ175.6 (NC(=O)), 103.5 (C-1_D, $J_{C,H}$ not determined), 102.1 (2C, C-1_B, 1_A, $J_{C,H}$ = 172 Hz), 101.2 (C-1_C, $J_{C,H}$ = 172 Hz), 98.4 (C-1_E, $J_{C,H}$ = 170 Hz), 80.1 (2C, C-2_B, 3_C), 79.5 (C-2_A), 76.5 (C-4_D), 75.7 (C-4_C), 74.4 (C-3_D), 73.4 (C-3_E), 73.0 (C-4_A), 72.9 (C-4_B), 72.6 (C-5_E), 72.0 (C-2_E), 70.8 (C-2_C), 70.5 (4C, C-3_A, 3_B, 4_E, 5_D), 70.1 (2C, C-5_A, 5_B), 69.4 (C-5_C), 61.4 (2C, C-6_D, 6_E), 56.6 (C-2_D), 55.7 (OCH_3), 23.1 (C(=O)CH_3), 18.7 (C-6_C), 17.5 (C-6_A*), and 17.3 (C-6_B*); FABMS for $C_{33}H_{57}NO_{23}$ (M, 835.33) *m/z* 858.6 $[M+Na]^+$.

Anal. Calcd for $C_{33}H_{57}NO_{23}$+3H_2O: C, 44.54; H, 7.14; N, 1.57%. Found: C, 44.78; H, 6.87; N, 1.84%. Compound **5** could not be obtained solvent-free.

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